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14. ABSTRACT
This project is designed to dissect out the primary event that initiates the alteration of epithelial cell homeostasis in inflammatory bowel disease (IBD). Our hypothesis is that activation-induced cytidine deaminase (AID, a DNA-modifying enzyme), which is ectopically expressed in epithelial cells only under intestinal inflammatory condition, is primarily responsible for the initiation of epithelial homeostatic alteration through epigenetic modification. During the past budget period, we have successfully developed and expanded two key mouse strains that are necessary for testing our hypothesis. One mouse strain is a fate-mapping double reporter mouse system that allows us to closely examine epithelial cells with prior AID expression versus those without it. By utilizing this mouse system, we have picked up a signaling transducers and activators of transcription 3 (STAT3) as a candidate molecule that is targeted by AID. Another mouse strain is AID-deficient recombination activation gene (RAG) 1 double knockout mice that allow us to determine the functional role of AID in the pathogenesis of IBD.

15. SUBJECT TERMS
activation-induced cytidine deaminase, epithelial cell homeostasis, Inflammatory bowel disease

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INTRODUCTION:
Inflammatory bowel disease (IBD) including Crohn's disease (CD) and ulcerative colitis (UC) is a chronic intestinal disorder that is caused by dysregulated host/microbial interactions (1,2). Intestinal epithelial cells (IECs), which provide the first line of defense against enteric microorganisms, are responsible for maintaining the appropriate host/microbial interactions (3). Despite the rapid turnover, an intact layer with a constant number of epithelial cells is maintained under healthy steady state. In contrast, the epithelial homeostasis is disrupted in IBD (4). Although many cytokines and growth factors have been demonstrated to participate in the alteration of epithelial homeostasis under intestinal inflammatory conditions, the primary event involved in the "initiation" of this alteration still remains unknown. In this project, we hypothesize that a DNA enzyme, activation-induced cytidine deaminase (AID), which can be ectopically expressed by epithelial cells under inflammatory conditions, is responsible for the initiation of epithelial homeostatic alteration through modification of chromatin status to enhance the accessibility of major transcriptional factors to their promoter regions. The AID-dependent alteration of epithelial homeostasis may contribute to the exacerbation of IBD. This grant proposal is designed to develop robust preliminary data that can be used as a foundation for future research projects to fully prove our hypothesis.

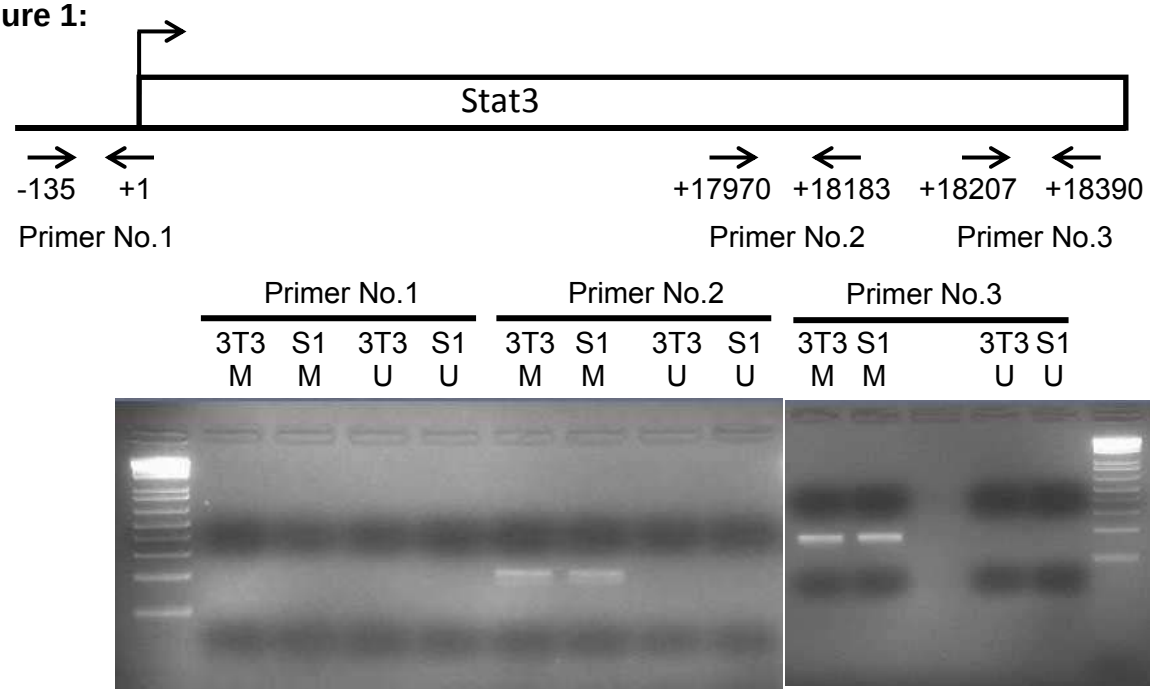
BODY:
Task 1: To investigate whether AID modifies the DNA methylation profiling and the chromatin accessibility in epithelial cells under intestinal inflammatory condition (months 1-18).

1a will expand mouse colony of KI/+ and KI/KI mice (months 1-3):
This 1a was designed to generate a double fluorescent Cre reporter knockin mouse system. In this system, cells express red fluorescent protein, and the color can be permanently changed to green once activation-induced cytidine deaminase (AID) is expressed (5,6). Therefore, this system allows us to distinguish epithelial cells with prior history of AID expressions from those without prior AID expression. As proposed in our original application, we have successfully established and expanded the two reporter mouse strains that express functional AID (KI/+) or non-functional AID (KI/KI).

1b. will correct DNA from epithelial cells from KI/+ mice (20 mice) and KI/KI mice (20 mice) (months 4-6):
By employing a 30mM EDTA perfusion method that allows us to isolate colonic epithelial cells as a crypt unit (7), we have successfully established a technique to purify red fluorescent+ epithelial crypts and green fluorescent+ epithelial crypts separately from a same individual colon. We confirmed that the purification method can preserve their viability during our experimental procedure. Through this method, DNA have been collected from the purified epithelial cells of KI/+ mice (carrying functional AID) and KI/KI mice (carrying non-functional AID) before and after induction of colitis by oral administration of dextran sulfate sodium (DSS).

1c will perform methylation-specific PCR (MSP) and EpiTect Methyl qPCR arrays and evaluate the data for future grant (months 7-18):
We have initiated the experiment using the methylation-specific PCR to see whether DNA methylation profiling is altered depending on the expression of AID. In this initial study, we first focused on some molecules involved in the epithelial cell proliferation. Unexpectedly, we found that the majority of these molecules proposed in our original application had already been demethylated in epithelial cells before AID expression. Alternatively, the promoter locus of STAT3 (signaling transducers and activators of transcription 3) was methylated in the epithelial cells before AID expression (Figure 1). Therefore, we are currently testing whether the methylated promoter of STAT3 gene in colonic epithelial cells is demethylated through an AID-dependent manner under intestinal inflammatory condition that is induced by oral administration of DSS. These data will be further confirmed using EpiTect Methyl qPCR arrays. In addition, as proposed in our original application, we will examine the methylation profiling of other molecules involved in the apopotosis/cell survival and host defense.

Figure 1:



Methylation-specific PCR was performed using bisulfite-treated DNA from CpG methylated NIH 3T3 mouse genomic DNA (3T3, as positive control of methylated STAT3) and from normal colonic epithelial cells without AID expression (S1). Methylated (M), but not unmethylated (U), bands were detected when CpG islands of STAT3 promoter regions were amplified using primers No.2 and No.3.

Task 2: To investigate the role of ectopic AID expression by epithelial cells in colitis (months 1-18)

2a will develop and expand the mouse colony of RAG1-deficient KI/KI mice (months 1-6).
In order to test the role of AID in the pathogenesis of colitis, we proposed to use a CD45RB model in which colitis is induced in recombinant activation gene (RAG)1 knockout mice versus AID-deficient (KI/KI) RAG double knockout mice by adoptive transfer of purified $CD4^+$ $CD45RB^{high}$ T cells from the spleen of WT mice. To generate AID-deficient (KI/KI) RAG double knockout mice, we crossed RAG1 knockout mice with AID knockout mice and have successfully developed small colony of AID-deficient RAG1 double knockout mice during the past budget period.

2b and c will purify $CD4^+$ $CD45RB^{high}$ T cells from the spleen of WT mice (60 mice) and transfer them into RAG1-deficient KI/KI mice (15 mice) versus RAG1-deficient KI/+ mice (15 mice) (months 7-12) and analyze the development of colitis in the recipient mice (months 9-15)
We recently transferred $CD4^+$ $CD45RB^{high}$ T cells from the spleen of WT mice into RAG1 knockout mice versus the newly generated AID-deficient RAG1 double knockout mice. Since the colitis develops in the recipient around 8 weeks after adoptive transfer, these recipient mice will be sacrificed 5 week later and the severity of colitis will be evaluated by the combination of macroscopic and microscopic examinations as proposed in our original application.

KEY RESEARCH ACCOMPLISHMENTS:
1. Establishment of mouse colony for double reporter mouse system that allows us to purify epithelial cells with a prior history of AID expression versus those without it from a same individual mouse
2. Establishment of a technique necessary to purify these two epithelial cell groups as a crypt unit
3. Identification of STAT3 as a potential target of AID
4. Establishment of AID-deficient RAG double knockout mouse colony to dissect out the role of AID in the pathogenesis of inflammatory bowel disease (IBD)

REPORTABLE OUTCOMES:
Two mouse strains: fate-mapping double reporter mouse strain and AID-deficient RAG1 double knockout mouse strain

CONCLUSION:
This project is to test whether a DNA enzyme AID is involved in the pathogenesis of inflammatory bowel disease through controlling the epigenetic modification in colonic epithelial cells. During the current budget period, all tools (including double reporter mice and AID-deficient RAG1 knockout mice) necessary to test our hypothesis have been successfully prepared. In addition, we have picked up STAT3 as a potential candidate molecule targeted by AID. We believe that our study, if fully completed, would provide novel insight into how epithelial cell homeostasis is altered in IBD and whether the alteration plays any roles in the pathogenesis of this disorder.

REFERENCES:
1. Abraham C, Cho JH. Inflammatory bowel disease. N Engl J Med. 2009;361:2066-78. PMID: 19923578
2. Xavier RJ, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. Nature. 2007;448:427-34. PMID: 17653185
3. Sartor RB. Key questions to guide a better understanding of host-commensal microbiota interactions in intestinal inflammation. Mucosal Immunol. 2011;4:127-32. PMID: 21248723
4. Dignass AU. Mechanisms and modulation of intestinal epithelial repair. Inflamm Bowel Dis. 2001;7:68-77. PMID: 11233665
5. Muzumdar MD, Tasic B, Miyamichi K, Li L, Luo L. A global double-fluorescent Cre reporter mouse. Genesis. 2007;45:593-605. PMID: 17868096
6. Robbiani DF, Bothmer A, Callen E, Reina-San-Martin B, Dorsett Y, Difilippantonio S, Bolland DJ, Chen HT, Corcoran AE, Nussenzweig A, Nussenzweig MC. AID is required for the chromosomal breaks in c-myc that lead to c-myc/IgH translocations. Cell. 2008;135:1028-38. PMID: 19070574
7. Hokama A, Mizoguchi E, Sugimoto K, Shimomura Y, Tanaka Y, Yoshida M, Rietdijk ST, de Jong YP, Snapper S, Terhorst C, Blumnerg RS, Mizoguchi A. Induced reactivity of intestinal $CD4^+$ T cells with an epithelial cell lectin, galectin-4, contributes to exacerbation of intestinal inflammation. *Immunity* 2004, 20:681-693. PMID:15189734

APPENDICES: None